

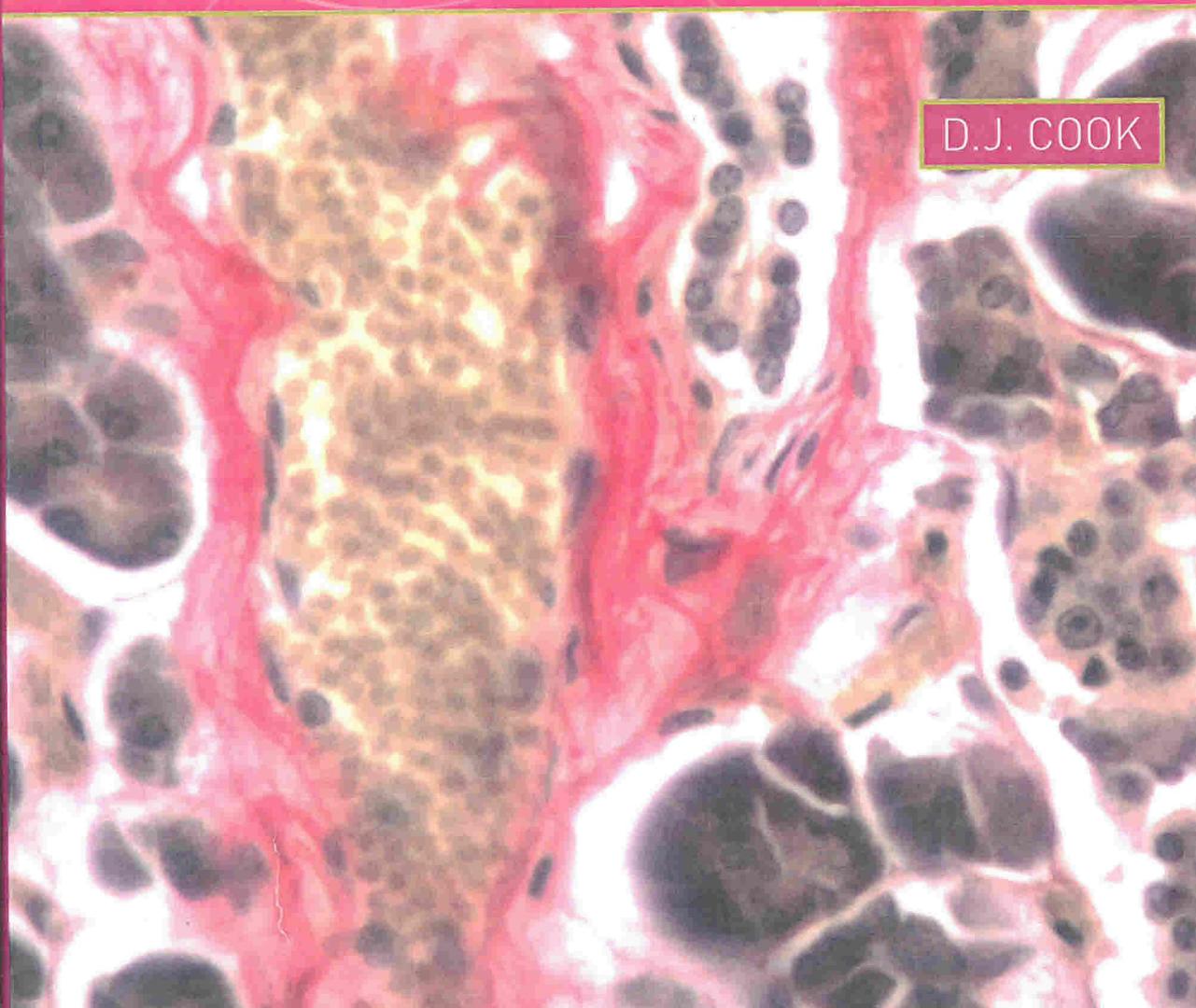
EXHIBIT O

CELLULAR PATHOLOGY

2nd EDITION:

AN INTRODUCTION TO TECHNIQUES
AND APPLICATIONS

D.J. COOK



CELLULAR PATHOLOGY

2nd edition

*Introduction to techniques and
applications*

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If sections of human tissue are examined under the microscope immediately after sectioning, they appear very dull and uninteresting. The tissue lacks contrast because all of the fixed materials have a similar refractive index and a similar colour so that a dull grey colour is all that can be seen. To bring out the structure of the tissues, it is essential to stain the cells to see the different parts in contrasting colours.

Staining is not simply random colouring of the sections but depends on using differences in the chemistry of the tissue to show the various components in different colours. This is most commonly done using dyes that can bind to the tissue in a selective way. Thus, the colours that are seen reflect the nature of the tissue and are not just a pretty picture. By using two or more dyes, it is possible to bring out the different materials in several contrasting colours.

The commonest stain in use is the haematoxylin and eosin (H&E) stain, which colours the nuclei a dark blue or purple and stains the cytoplasm and connective tissue in shades of pink (see *Colour plate 5*).

6.1 STAINING MECHANISMS

The binding of dyes to tissues is no different to any other chemical bonding and the mechanisms rely on the same binding forces that occur in all other organic compounds. The dye must form some type of bond or link to the tissue or they will simply be rinsed out of the tissue when the section is washed in another reagent. The usual forms of bonding can be involved. Each type has its own characteristics and bond strengths.

<i>Bond type</i>	<i>Strength (kcal mole⁻¹)</i>
Ionic bonds	40–110
Hydrogen bonds	2–7
Van der Waals forces	1–2
Covalent bonds	35–212
Hydrophobic interactions	4–8

Ionic bonding

Ionic bonding involves electrostatic attraction between oppositely charged ions. One ion is a fixed ion in the tissue section and the other is the dye ion. Anionic (negatively charged) dyes will bind to cations (positively charged) in the tissue, and cationic dyes will bind to tissue anions.

Ionic bonding is the single most important form of bonding in most histological staining. Almost all simple staining can be understood and controlled by understanding the ionic charges involved.

Negatively charged eosin ions will stain positively charged tissue ions

Eosin is an example of an anionic dye and is attracted to protein groups that are positively charged (cations) such as amino groups. First, the amino